Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6447–6450, October 1984
Genetics

Genomic constitution of an H-2:Tla variant leukemia

(immunoselection/*H-2:Tla* deletion/mitotic recombination)

F. W. SHEN*, R. S. K. CHAGANTI*, L. A. DOUCETTE*, G. W. LITMAN*, M. STEINMETZ†, L. HOOD‡, AND E. A. BOYSE*

*Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; †The Basel Institute for Immunology, 487 Grenzacherstrasse, Basel, Switzerland; and ‡Division of Biology, California Institute of Technology, Pasadena, CA 90025

Contributed by Edward A. Boyse, June 28, 1984

ABSTRACT **A TL^+ leukemia of a (B6 × A)F_1 hybrid mouse (*H-2*^{b}/*H-2*^{a}) was previously subjected to immunoselection against H-2^a by passage in (B6 × A.SW)F_1 mice (*H-2*^{b}/*H-2*^{s}). A variant leukemia line was obtained that serologically lacked not only the H-2^a phenotype but also the TL phenotype determined by the linked *cis Tla*^{a} allele of strain A. The H-2^b phenotype and the TL phenotype of the *Tla*^{b} allele of the B6 strain, which is expressed only by leukemia cells, were retained by the variant. Southern blotting with an H-2 cDNA probe that identifies restriction fragment polymorphisms distinguishing alleles of the *H-2* and *Tla* regions of the B6 and A strains indicates that both the *H-2*^{a} and *Tla*^{a} alleles are missing from the genome of this H-2^a:Tla^a negative variant. Since the variant has two apparently unaltered chromosomes 17, where the *H-2:Tla* complex is situated, and since the intensity of bands in Southern blotting is suggestive of *H-2*^{b} homozygosity, it is considered that loss of the *H-2*^{a}:*Tla*^{a} haplotype by the variant was accompanied by duplication of the *H-2*^{b}:*Tla*^{b} haplotype. The implied change from heterozygosity to homozygosity that the variant has undergone with respect to *H-2:Tla* was not paralleled by a similar change at the three other loci tested, since the variant retained heterozygosity for *Pep-3* (chromosome 1), *Gpi-1* (chromosome 7), and *Es-1* (chromosome 8).**

When *H-2* heterozygous tumor cells of the mouse are subjected to immunoselection against the products of one of their H-2 phenotypes, variant cell lines are obtained that lack that H-2 phenotype (reviewed in ref. 1).

As a means to understanding the relation of leukemia to the *Tla* locus, which exhibits abnormal expression in TL^+ leukemia cells of TL^- mouse strains like C57BL/6 (B6), a variant of this kind was previously selected from a TL^+ (B6 × A)F_1 hybrid leukemia (H-2^b/H-2^a) by passage in (B6 × A.SW) recipients (H-2^b/H-2^s) and was found to have lost not only the serological H-2^a phenotype but also the TL phenotype determined by the linked (*cis*) *Tla*^{a} allele (2). The serological phenotypes for H-2^b and Tla^b were retained. It has now been possible to study this phenotypically negative variant further to determine whether it has undergone genomic loss of the *H-2* and *Tla* regions of the *H-2*^{a}:*Tla*^{a} haplotype.

MATERIALS AND METHODS

Preparation of the Probe. Probe IIa (3) was isolated from the pH-2IIa clone by digestion with *Pst* I and *Pvu* II or with *Hha* I and *Sac* I, followed by self-ligation with T4 ligase. The probe was nick-translated to a specific activity of ≈2 × 10^8 cpm/μg with [α-^{32}P]dNTP.

Preparation of DNA. High molecular weight DNA was prepared from normal thymocytes and spleen cells combined or from leukemia cells according to Blin and Stafford (4), except that the cells were not frozen and that two further extractions with chloroform/isoamylalcohol (24:1) were performed after phenol extraction.

Table 1. Phenotypes

Phenotype	Cell: (B6 × A)F_1 thymocyte	Cell: (B6 × A)F_1 leukemia, Unselected	Cell: (B6 × A)F_1 leukemia, Variant
H-2^a	+	+	−
H-2^b	+	+	+
TL.1	+	+	+
TL.2	+	+	+
TL.3	+	+	−
TL.4	−	+	+

The variant was selected by passage in (B6 × A.SW)F_1 hybrids (2).

DNA Blot Hybridization. DNA was completely digested with restriction enzyme *Eco*RI, *Bam*HI, or *Bgl* II, subjected to electrophoresis on 6-mm thick 0.7% agarose gels in TAE buffer (40 mM Tris acetate/2 mM EDTA, pH 8.0), and transferred to nitrocellulose filters. The blots were baked and hybridized with ^{32}P-labeled pH-2IIa probe as described (5).

Phenotypes of the Unselected and Variant Leukemia Lines. This information is given in Table 1. The unselected and variant leukemia lines were carried in passage in ascites form in (B6 × A)F_1 hybrids or in culture if so indicated.

Cytogenetics. Cytogenetic studies were performed on actively proliferating cultures of the unselected and variant leukemia cell lines. Colcemid (0.05 μg/ml) was added to cultures 1 hour before harvest. Chromosome preparations were made by conventional methods using 0.075 M potassium chloride as the hyposmotic solution and 3:1 methanol/acetic acid as the fixative. Q-banding was used in the analysis of karyotypes (6).

RESULTS

Southern Blotting with the pH-2IIa Probe. The pH-2IIa probe was hybridized with *Eco*RI restriction fragments (RFs) of DNA from cells of the unselected and H-2^a:Tla^a negative variant leukemia lines. The RF patterns are shown in Fig. 1 in comparison with cells of the B6 (*H-2*^{b}:*Tla*^{b}) and A (*H-2*^{a}:*Tla*^{a}) parental inbred strains and of the B6-*Tla*^{a} and A-*Tla*^{b} recombinant congeneic strains. Use of B6-*Tla*^{a} and A-*Tla*^{b} congeneic cells (7, 8) permits assignment of RF polymorphisms to the *H-2:Qa-2,3* and telomeric *Tla* regions. The RF patterns of unselected (B6 × A)F_1 leukemia cells and of normal (B6 × A)F_1 cells were not distinguishable from one another and composed a combination of RFs of the B6 and A parental inbred strains. The RF pattern of the H-2^a:Tla^a

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Abbreviation: RF, restriction fragment.

§Typed by Harold A. Hoffman of Animal Genetic Systems (11 Taft Court, Rockville, MD 20850).

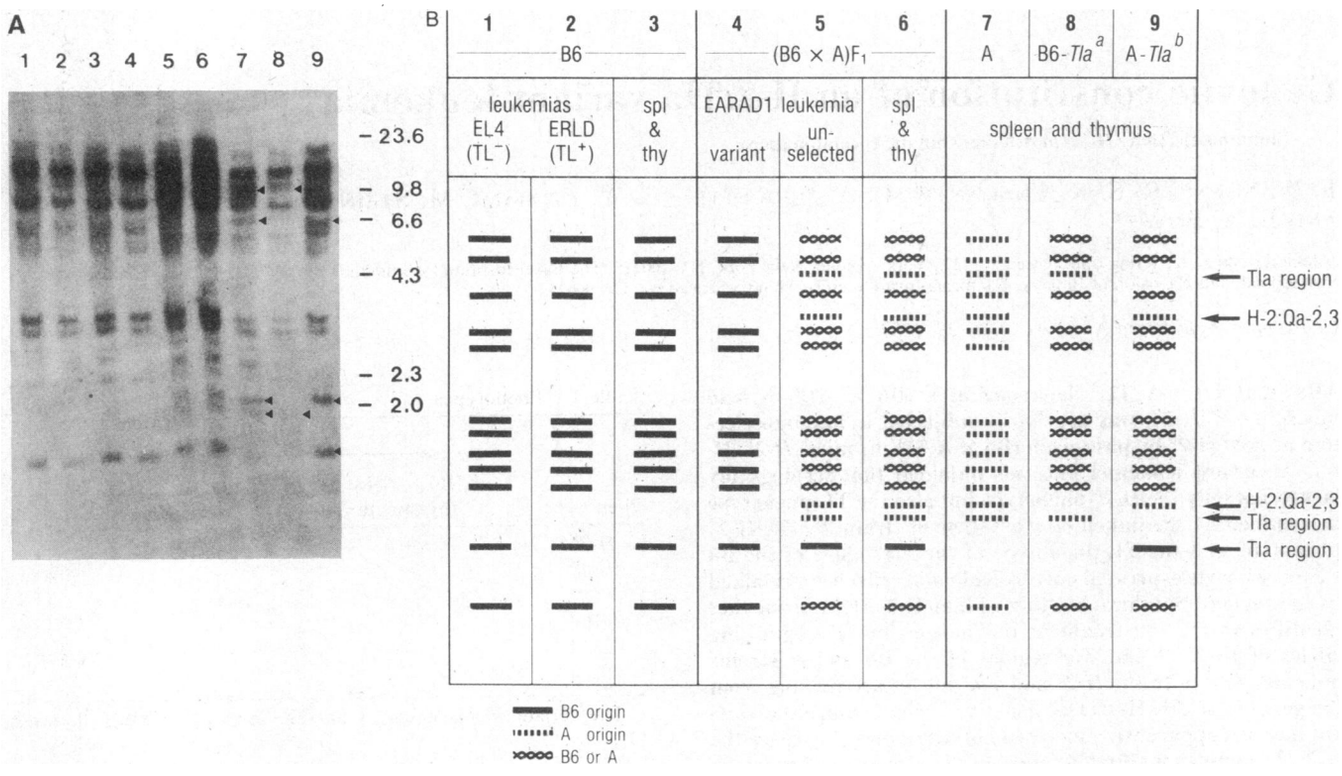

FIG. 1. Southern blots of *Eco*RI restriction fragments (shown in kilobases) hybridized with the pH-2IIa probe, showing one of several exposure times needed (*A*) to visualize both faint and strong polymorphic fragments, and key diagram (*B*). The quantity of DNA digested with 10 μg for all cells except those of the unselected leukemia (lane 5) and (B6 × A)F_1 normal cells (lane 6), for which 20 μg was used, provides equal material for showing RF polymorphisms. The point of recombination for the B6-*Tla*^{a} haplotype is between *Qa-2,3* and *Tla* (7, 8). Similar results were obtained with *Bam*HI and *Bgl* II.

negative variant, on the other hand, was indistinguishable from that of B6 normal cells and of B6 leukemia cells. The patterns were not affected by increase in the concentration of restriction enzyme. Accordingly, four polymorphic RFs of A strain origin are missing from the variant, and another of B6 (*Tla* region) origin is retained. The intensity of this B6 band from the variant cells was similar to B6 (*Tla*^{b}) and A-*Tla*^{b} cells for equal input of DNA and to twice the amount of (B6 × A)F_1 DNA, suggesting that the variant cells are now homozygous in the *Tla* region.

Results similar to those described above were obtained with *Bam*HI and *Bgl* II, both of which, like *Eco*RI, identify RF polymorphisms in both the *H:2*:*Qa-2,3* and *Tla* regions.

Biochemical Phenotypes§. Pep-3 (chromosome 1), Gpi-1 (chromosome 7), and Es-1 (chromosome 8) are three biochemical markers for which the B6 and A strains differ and which were expressed in the leukemia cells. For all three markers, the unselected and variant lines displayed the phenotype of heterozygotes. DNA from host cells contaminating the washed ascites leukemia cells used for these tests can be discounted because such contaminant cells are very scarce and because the intensity of reaction was the same for the unselected and variant cell lines.

Cytogenetics. Q-banded chromosomes were examined from 50 metaphase spreads, each from the unselected and the variant preparations (Fig. 2). The unselected cells exhibited a modal chromosome number of 40 (45 cells) and a normal male karyotype (40, XY). Both chromosomes 17 exhibited a normal banding pattern. One hypodiploid cell (33 chromosomes) and 1 hyperdiploid cell (60 chromosomes) were presumed to be technical artefacts. A long acrocentric chromosome (mar 1) was present in 2 cells (41 chromosomes), whereas the final cell, which had mar 1, also had an additional tiny acrocentric chromosome (mar 2). The latter 3 cells therefore belong to a clone, making the population a mosaic

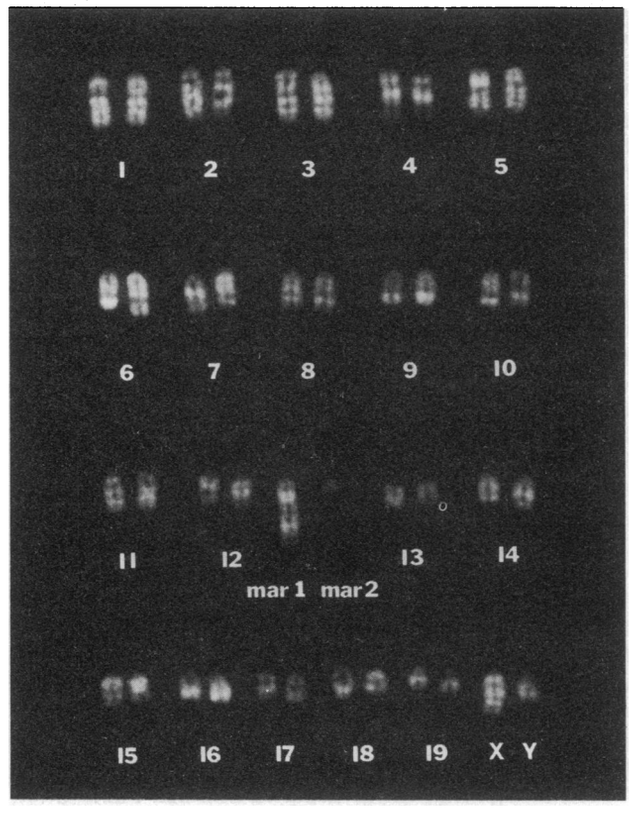

FIG. 2. Q-banded karyotype of a presumed *H-2*^{b}:*Tla*^{b} homozygous cell of the variant line showing the two marker chromosomes. The karyotype otherwise is normal. (×1300.)

one. The mar 1 chromosome comprised a tandem duplication of chromosome 12 with the segment 12B → ter attached to another chromosome 12 at band F2. The banding pattern of mar 2 was compatible with that of the segment 12cen → 12B. Since each of these cells also had 2 normal chromosomes 12, the markers probably were derived through an inter-chromatid exchange between the 2 chromosomes 12 followed by non-disjunction in the progenitor cell. Loss of mar 2, probably again by non-disjunction, resulted in the clone with 41 chromosomes. Thus, the cells with 42 chromosomes are tetrasomic for the entire chromosome 12, whereas those with 41 chromosomes are tetrasomic for the region 12B → F and disomic for the regions 12cen → 12B and 12F → ter. Of the 50 variant cells studied, 11 had 42 chromosomes, each with mar 1 and mar 2, whereas the remaining 39 had 41 chromosomes, each with mar 1 alone. The karyotype of these cells, including both chromosomes 17, was otherwise normal. Therefore, the selection applied must have fixed a 42-chromosome cell with mar 1 and mar 2, which also became homozygous at the *H-2:Tla* region as the progenitor of the variant. The unstable mar 2 was lost in the majority of unselected as well as variant cell populations.

DISCUSSION

A feature of special interest concerning H-2:Tla loss variants, which prompted investigation of TL phenotypic losses by H-2-selected variants in the first place (2), is the possible relation of *Tla* genes to malignant transformation. The main point is that TL antigens are expressed not only by T-cell leukemias of mice whose thymocytes express TL constitutively (Tla^a; e.g., strain A) but also by T-cell leukemias of TL-null mice whose thymocytes do not express TL (Tla^b; e.g., strain B6)—hence, the choice of a (B6 × A)F_1 leukemia, which expresses both constitutive (Tla^a) and irregular (Tla^b) TL antigens, in determining whether retention of particular *Tla* alleles is required for the proliferative behavior of TL^+ leukemias. We now see that this is not so for the constitutive Tla^a allele of the leukemia in question.

Whatever genomic abnormality there may be in the Tla^b locus retained by the variant, which exhibits abnormal expression in leukemia cells, it was not revealed in the present study since the RF banding pattern of the unselected leukemia cells did not demonstrably differ from that of normal (B6 × A)F_1 cells nor did the RF patterns of the variant cells and of TL^+ and TL^- B6 leukemia cells demonstrably differ from that of normal B6 cells.

A reciprocal ($H\text{-}2^b$:Tla^b negative) variant was previously selected from the same leukemia by passage in (A × B6-$H\text{-}2^k$)F_1 recipients ($H\text{-}2^a/H\text{-}2^k$) and its proliferative capacity also was not changed (9). Unfortunately, this variant did not survive frozen storage, and it is unsafe to assume that it also arose by genomic loss of an *H-2:Tla* haplotype. Immunoselection for loss of both *H-2* haplotypes has never succeeded with tumors of any kind [loss due to a β2M mutation is another matter since this does not involve genomic loss of H-2 (10, 11)] and the question remains whether a change at both *Tla* loci may be obligatory in leukemic cellular proliferation.

With regard to mechanisms of variant formation, there is no reason to suppose that they are peculiar to transformed cells, though much more difficult to study in normal somatic cells. Somatic segregation and mitotic recombination were originally favored in the case of H-2 loss variants because it was often possible to select partial loss variants of $H\text{-}2K^-$:$H\text{-}2D^+$ type with respect to one haplotype but rarely or never the $H\text{-}2K^+$:$H\text{-}2D^-$ type ("polarity") (1). This mechanism seemed to be ruled out when it was later found that the centromere of chromosome 17 lies on the *K* rather than the *D* side of *H-2*, but Susumu Ohno (personal communication) points out that somatic recombination accompanied by reduction and subsequent duplication of chromosome 17 would give the *K–D* polarity actually observed.

More generally, likely cytogenetic mechanisms that can account for the formation of variants of the sort in question are the following: (*i*) *hemizygosity*, attained either by deletion of a chromosome segment or by chromosome loss from non-disjunction, and (*ii*) *homozygosity*, attained by point mutation, chromosome loss by non-disjunction followed by duplication of the remaining chromosome by a second non-disjunction, or mitotic recombination. Monosomy is relatively common in human leukemia cells (12); indeed, in a subset of acute lymphoblastic leukemia the entire autosomal complement may become monosomic, giving rise to near-haploid genomes (13, 14). Cytological evidence for mitotic recombination in human lymphoid and other cells has been presented by several investigators during the past 20 years (15–18). In retinoblastoma, a childhood intra-ocular tumor with a predisposing gene on chromosome 13 (*Rb-1*), a recent comparison of constitutional and tumor genotypes at a number of chromosome 13 loci (*esterase-D*, a gene closely linked to *Rb-1* and several RF length polymorphisms related to arbitrary loci) showed that tumorigenesis is associated with hemizygosity attained by non-disjunctional loss of the chromosome carrying the wild-type allele or homozygosity attained either by duplication of the chromosome carrying the mutant allele in a hemizygous cell or by mitotic recombination of *Rb-1* (19, 20). An identical situation has just been reported at several loci on chromosome 11p (restriction length fragment polymorphisms flanking the genes for parathyroid hormone, β-globin, insulin, and c-Ha-*ras*-1) when constitutional genotypes were compared with tumor genotypes in a number of patients with Wilms tumor, a childhood kidney tumor with a predisposing locus on 11p (21–24).

In the present study, analysis of both restriction enzyme fragments and chromosomes indicated that loss of the $H\text{-}2^a$:Tla^a haplotype by the variant was accompanied by duplication of the $H\text{-}2^b$:Tla^b haplotype. Rajan *et al.* (25) recently isolated $H\text{-}2^b$ and $H\text{-}2^d$ loss variants from an $H\text{-}2^b/H\text{-}2^d$ heterozygous Friend leukemia cell line by immunoselection with antisera against the respective haplotypes. Their studies of restriction enzyme fragments and alleles at the *H-2*-linked locus *Glyoxylase-1* (*Glo-1*) also implied attainment of homozygosity for the remaining H-2 type following selection. Those results and ours are consistent with an origin of homozygosity by mitotic recombination, although formally neither set of data permits distinction between non-disjunctional loss of a chromosome followed by duplication of its homologue and mitotic recombination. Certainly the length of chromosome rendered homozygous can extend from unselected loci on the centromeric side of *H-2* (*Glo-1*) to unselected loci on the telomeric side (*Tla*). Thus, loci to which no selection pressure has been applied may be rendered homozygous if they are linked to the immunoselected *H-2* complex.

Events at other heterozygous loci that are not *H-2* linked are of interest because they bear on the question of whether the variant-producing mechanism affected only chromosome 17 or the genome as a whole. For instance, if all chromosome pairs were involved, as in meiotic segregation and recombination, then there is a 50% chance of homozygosity at any originally heterozygous locus not linked with *H-2* and situated 50 centimorgans or more from the centromere of that chromosome. For loci nearer the centromere the chance would be proportionally higher. The fact that the three loci, *Pep-3*, *Gpi-1*, and *Es-1* (chromosomes 1, 7, and 8), remained heterozygous in the variant would suggest that the event that produces an *H-2:Tla* homozygous variant in the circumstances described characteristically affects a single chromo-

some pair—in this case, chromosome 17—particularly since *Gpi-1* is probably <15 centimorgans from its centromere.

The marker chromosomes observed are of interest in another context. The distal breakpoint in mar 1 is in band 12F2 at which chromosome 12 also rearranges in one of the two consistent translocations reported in Pristine-induced plasmacytomas in BALB/c mice—namely, t(12;15)(F2;D3) (26). In this translocation, the cellular oncogene *myc*, normally located on chromosome 15, is translocated into the immunoglobulin heavy chain (*IgH*) locus, whose position previously has been determined to be distal to band 12F1 (27). The rearranged c-*myc* has been shown to undergo activation (28). A parallel translocation of human c-*myc*, normally located at 8q24 (29) to the *IgH* locus, at 14q32 (30) has also been shown to lead to c-*myc* activation in Burkitt lymphoma (31, 32). In the human autosomal recessive disorder ataxia-telangiectasia (A-T), a primary immunodeficiency that strongly predisposes to lymphoid neoplasia, a translocation occurring in the T lymphocytes of a number of patients involves the 2 chromosomes 14 with breaks in a proximal (q12) and a distal band (14q23) (33); the latter, as noted above, is in the region *IgH*. A-T lymphocytes with the 14-14 translocation exhibit proliferative advantage and eventually become the only recognizable T cells in the peripheral circulation (34). Possibly the rearrangement causes activation of a T-cell proliferation-related gene normally situated at 14q12. As with the 14-14 translocation in A-T, the 12-12 translocation observed in the $H\text{-}2^a$ loss variant also may transpose a proximal gene into the distal *IgH* locus on chromosome 12. The significance of this rearrangement in the evolution of the class of leukemias represented in the present study remains to be determined.

This work was supported in part by Grants CA-22131, CA-20473, and CA-34775 from the National Institutes of Health. E.A.B. is American Cancer Society Research Professor of Cell Surface Immunogenetics.

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